REMARKS

Entry of the foregoing and further and favorable consideration of the subject application are respectfully requested.

As correctly stated in the Official Action, claims 1-25 are pending in the application. Claim 24 stands withdrawn from consideration. Claims 1-23 stand rejected.

By the present amendment, a substitute specification has been amended to correct the issues pointed out by the Examiner in the Official Action, including typographical errors, addition of SEQ ID NOs, and reference to Figure 16. A marked up copy of the specification is also attached hereto. No new matter has been added.

By the present amendment, the claims have been amended to more precisely define the presently claimed invention. Specific amendments of formalities are discussed below with regard to the objections and rejections. No new matter has been added.

Claims 2 and 6 have been canceled. Claim 1 has been amended to incorporate the elements of Claim 6. Claim 9 has been amended to more precisely define the invention. Support for the amendment to Claim 9 is found on, at least, page 6, lines 2 through 8. Accordingly, no new matter has been added. The amendment to Claims 10, 12, 19, 21, and 23 is supported, at least, by the originally filed specification on page 13, line 26 to page 14, line 8.

New Claims 26, 27, and 28 have been added. Support for these claims can be found, at least, in claim 9 as originally filed, and on page 13, line 16 to page 14, line 8.

Lack of Unity of Invention

Claim 24 now recites "a polynucleotide encoding the RNA polymerase of Claim 1." Therefore, this claim shares the same technical feature of the claims of Group I (Claims 1-23 and 25), mutations which allow the incorporation of 3'-deoxyribonucleotides. Consideration of this amended claim is respectfully requested.

Drawing Objections

The multiple views of figures 16 and 18 objected to on form PTO-948 have been relabeled in accordance with 37 C.F.R. § 1.84(h).

Objections to the Specification

The specification has been amended as suggested by the Examiner on pages 4-5 of the Official Action using a substitute specification.

Claim Objections

Claims 1, 3, 7, and 10 stand objected to for various informalities. Without conceding to the merits of these objections and solely in an effort to expedite prosecution, these claims have been amended as suggested by the Examiner on page 6 of the Official Action or with a comparable amendment. No new matter has been added.

Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-23 and 25 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite.

A. Claims 1-8, 10, and 25 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite in the recitation of "3'-deoxyribonucleotides and derivatives thereof." This rejection, as it applied to the claims as amended, is respectfully traversed.

Applicants respectfully direct the Examiner's attention to the claims of U.S. Patent Nos. 6, 074,824 and 6,365,350 (attached hereto for the Examiner's convenience), which are patents of the Applicants for related inventions. Particularly, the Examiner's attention is directed to Claim 1 of both the '824 and the '350 patents, which contain language referring to "derivatives" of certain nucleotides. Under 35 U.S.C. § 282, U.S. patents are presumptively considered valid. Therefore, the present application, employing very similar language to these two patents, should also be deemed definite as what is encompassed is clearly understood by one skilled in the art.

The word "derivative" is well understood by the skilled artisan to encompass 3'-doexyribonucleotides carrying any base modification but still recognized by the RNA polymerase. The RNA polymerase according to the presently claimed invention has the ability to incorporate 3'-deoxyribonucleotides at a rate at least twice higher than the wide type RNA polymerase (specification page 10, lines 26-35). The mutated RNA polymerase has a higher ability to distinguish between ribonucleotides and 3'-deoxyribonucleotides, so that 3'-deoxyribonucleotides are recognized and introduced more efficiently. The

difference between a ribonucleotide and a 3'-deoxyribonucleotide is the presence of a hydroxyl group versus a hydrogen at position 3' of the sugar ring respectively. As a consequence, the essential characteristic for a 3'-deoxyribonucleotide to be incorporated by an RNA polymerase resides in the presence of the hydrogen at position 3'.

Thus, any derivatives of 3'-deoxyribonucleotide known in the art, *e.g.*, a modified base, or the addition of a label such as a fluorescent label, digoxygenin or radiolabel, which do not interfere with the incorporation of the 3'-deoxyribonucleotide would be encompassed within the presently claimed invention. Modified bases are well known in the art. For example, the Examiner's attention is directed to Table 13-3 of the U.S. Patent and Trademark's Office PatentIn version 2.1, User's Manual.

It is thus common knowledge that the term "derivative of 3'-deoxyribonucleotide" includes any modification different from the 3' position of the sugar and which can be recognized by the RNA polymerase. Accordingly, the claim language complies fully with 35 U.S.C. § 112, second paragraph. Withdrawal of this rejection is respectfully requested.

B. Claims 9 and 11-23 stand rejected as purportedly indefinite in the recitation of amino acid residues without a reference sequence. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, these claims have been amended to recite specific SEQ ID NOs which correspond to the particular phage recited in the claim. No new matter has been added. Accordingly, withdrawal of this rejection is respectfully requested.

C. Claims 2-5 stand rejected as allegedly indefinite in the recitation of "a nucleotide binding site of a wild type polymerase. Claim 2 has been canceled, thereby mooting the rejection with regard to this claim. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, Claims 3, 4, and 5 now depend either directly or indirectly from Claim 1 which does not contain the rejected claim language. No new matter has been added. Accordingly, withdrawal of this rejection is respectfully requested.

D. Claims 8, 9, 11, and 18-23 stand rejected based on the recitation of "derived from" T7, T3, SP6, or K11 phages. Without conceding to the merits of this rejection, and solely in an effort to expedite prosecution, these claims have been amended to omit the term "derived" as suggested by the Examiner on page 10 of the Official Action. No new matter has been added. Accordingly, withdrawal of this rejection is respectfully requested.

E. Claims 12, 15, 17, 19, 21, and 23 stand rejected as allegedly indefinite for failing to further limit the claim they depend from. Applicants respectfully traverse this rejection, as it applies to the claims as amended. The Examiner appears to have misinterpreted the claims. Claim 11 describes an RNA polymerase from T7 phage with a "tyrosine at amino acid residue 644 or 667 of SEQ ID NO:2." Claim 12 which depends from Claim 11 further limits Claim 11 by stipulating that the mutant RNA polymerase of Claim 11 has an additional "substitution, insertion, or deletion of an amino acid **other than** the amino acid residues 644 and/or 667 of SEQ ID NO:2 and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase

activity." Therefore, Claim 12 does contain additional limitations not present in Claim 11 and is a proper dependent claim. Claim 19 similarly limits Claim 18 for T3 phage, Claim 21 similarly limits Claim 20 for K11 phage, and Claim 23 similarly limits Claim 22 for SP6 phage.

Similarly, Claim 15, which depends from Claim 13, further limits Claim 13 by stipulating that the RNA polymerase of Claim 13, which **already contains** a Y644F mutation, also has the "665th amino acid residue, leucine, of SEQ ID NO:2 ... replaced with proline." Therefore, Claim 15 further limits Claim 13. Claim 17 similarly limits Claim 16.

Accordingly, as the rejected dependent claims properly add limitations to the claims they depend from, no additional amendments as suggested by the Examiner on page 10 of the Official Action are believed necessary. Withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-10, 12, 19, 21, 23, and 25 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter not described in the specification in such a way as to reasonably convey to the skilled artisan that the inventors had possession of the claimed invention at the time the invention was filed. The Examiner has rejected the claims as overly broad in the incorporation of all possible RNA polymerases with enhanced 3'-deoxyribonucleotide incorporating ability. The Examiner argues that only representative species are disclosed in the present application and that no identifying structural

characteristics or properties other than the activities recited in claim 1 (which he argues is unpredictable) are disclosed. This rejection, as it applies to the claims as amended, is respectfully traversed.

By the present amendment, Claims 1 and 9 have been amended to more precisely define the region in which the substantive modification has been made, *i.e.*, that which confers greater 3'-deoxyribonucleotide incorporating ability. These claims encompass the regions shown in Figure 5, in which a loop between helix Y and helix Z and a loop between helix Z and helix AA (Claim 1) and the amino acid region of 641-667 of SEQ ID NO:2 (claim 9). Furthermore, Claims 10, 12, 19, 21, and 23 are limited to further substitutions, insertions, or deletions which "do not substantially affect the RNA polymerase activity," *i.e.*, "silent" mutations. Thus, structural and functional characterization is contained within the claims.

Applicants respectfully submit that the method for amino acid modification or mutation and screening of mutants for such a small stretch of amino acids is very common in the art. For example, the Sousa *et al.* reference cited by the Examiner and the specification on page 11, line 22 to the end of page 18, show such a method. Preparation of mutants is a very common technique as of the filing date of this application and the screening of mutants with improved incorporation ability of 3'-deoxyribonucleotides is clearly explained such that the skilled artisan could undertake such activity. The region of modification is very limited and, based on the disclosure of the present application, there is a high degree of predictability to arrive at the present invention without undue experimentation.

The region in a loop between helix Y and helix Z and/or loop between helix Z and helix AA and the region 641-667 related to the binding site of RNA polymerase (specification, page 16), so that any modified amino acid within this region resulting in an RNA polymerase having an improved ability to incorporate 3'-deoxyribonucleotides is encompassed by the presently claimed invention. Applicants disclose in Table 1 (page 35 of specification) at least four modifications, *i.e.*, F644Y, F644Y/L665P, L665P, F667Y, and F644Y/L665P/F667Y. These mutations are within the region in a loop between helix Y and helix Z and/or a loop between helix Z and helix AA, and within region 641-667 and result in an RNA polymerase having an improved ability to incorporate 3'-deoxyribonucleotides compared to wild-type RNA polymerase.

Thus, Applicants have concretely demonstrated at least 4 RNA polymerase modifications within the region in a loop between helix Y and helix Z and/or a loop between helix Z and helix AA, and the in amino acids 641-667 which possess enhanced 3'-deoxyribonucleotide incorporating ability. This experimental proof coupled with the description contained in the specification provide the skilled artisan with the necessary tools and description to practice the presently claimed invention with predictability.

Applicants respectfully submit that they are not required to demonstrate all possible modifications of every amino acids within the region in a loop between helix Y and helix Z and/or a loop between helix Z, and amino acids 641-667. With the guidance provided by the specification and that well known in the art, the skilled artisan is more than capable of practicing the presently claimed invention without exhaustive experimental data submitted for every possible mutant RNA polymerase. As such, the claims meet the requirements of

35 U.S.C. § 112, first paragraph. Accordingly, withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 102(b)

Claims 1-3, 6-9, and 25 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Sousa *et al.* (*EMBO J.* 14(18):4609-4621 (1995)). The Examiner argues that Sousa et al. disclose a T7 RNA polymerase which uses deoxyribonucleotide triphosphates at a greater rate than ribonucleotide phosphates. This rejection, as it applies to the claims as amended, is respectfully traversed.

In order to anticipate a claim under 35 U.S.C. § 102(b), the cited publication must contain all of the elements of the claimed invention. Applicants respectfully submit that the Sousa *et al.* publication does not meet this burden.

Claim 1 of the presently claimed invention is directed toward an RNA polymerase having a mutation in an "amino acid present in a loop between helix Y and helix Z and/or a loop between helix Z and helix AA in the wild-type polymerase" such that the "ability ... to incorporate 3'-deoxyribonucleotides is enhanced." Independent claim 9 is directed toward an RNA polymerase with "at least one amino acid present ...corresponding to amino acid residues 641-667 of SEQ ID NO:2" modified to "enhance the ability of the RNA polymerase to incorporate 3'-deoxyribonucleotides" compared with wild-type RNA polymerase.

Applicants respectfully submit that Sousa *et al.* never disclose the use of 3'-deoxyribonucleotides by RNA polymerase, much less the enhanced ability of a mutated

RNA polymerase to incorporate them. The Sousa *et al.* publication solely deals with using the mutant RNA polymerase to synthesize RNA, DNA, or mixed dNMP/rNMP transcripts. Sousa *et al.* does not disclose the use of mutant RNA polymerases to sequence polynucleotides using 3'-terminators (3'-deoxyribonucleotides), but rather only the use of dNTPs (i.e., 2'-dNTPs) and rNTPs. As such, the Sousa *et al.* publication does not disclose the incorporation of 3'-terminator and thus, provide no disclosure regarding the enhanced ability of a mutant RNA polymerase to recognize and incorporate 3'-terminators.

As the Sousa *et al.* publication does not contain all of the elements of the presently claimed invention, it cannot anticipate the presently claimed invention under 35 U.S.C. § 102(b). Accordingly, withdrawal of this rejection is respectfully requested.

Allowable Subject Matter

Applicants gratefully acknowledge the Examiner's statement that claims drawn to an RNA polymerase comprising wild type phage RNA polymerase which has a mutation of amino acid residues 644, 665, or 667 of SEQ ID NO: 2 would be allowable.

Conclusions

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the application may be expedited.

Respectfully submitted,

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Attachment to REPLY & AMENDMENT dated May 20, 2002

Marked-up Claims 1, 3, 4, and 7-24

1. (Amended) An RNA polymerase consisting of a wild type RNA polymerase wherein at least one [of] amino [acids] acid present in a loop between helix Y and helix Z and/or a loop between helix Z and helix AA in the wild type RNA polymerase [is] has been modified to enhance [its] the ability of the wild type polymerase [for incorporating] to incorporate 3'-deoxyribonucleotides and derivatives thereof in comparison with the corresponding wild type RNA polymerase.

3. (Amended) The RNA polymerase of claim [2] 1, wherein the amino acid modification [of amino acid] is substitution, insertion or deletion of an amino acid.

4. (Twice Amended) The RNA polymerase of claim 1, wherein at least one amino acid present [in the nucleotide binding site] present in a loop between helix Y and helix Z and/or a loop between helix Z and helix AA of the wild type RNA polymerase is replaced with tyrosine.

7. (Twice Amended) The RNA polymerase of claim 1, which has been modified so that the ability of the RNA polymerase to incorporate [for incorporating] 3'-deoxyribonucleotides and derivatives thereof into a polynucleotide [should be] is increased by twice that of [in comparison with] the wild type.

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Marked-up Claims 1, 3, 4, and 7-24

8. (Twice Amended) The RNA polymerase of claim 1, [which] wherein the RNA polymerase is [derived] from T7 phage, T3 phage, SP6 phage, or K11 phage.

9. (Amended) An RNA polymerase consisting of a wild type RNA polymerase [provided that] wherein at least one [of] amino [acids] acid present in a region of the wild type RNA polymerase corresponding to amino acid residues 641-667 of SEQ ID NO:2 of RNA polymerase [derived] from T7 phage has been modified to enhance the ability of the RNA polymerase to incorporate 3'-deoxyribonucleotides and derivatives thereof into a polynucleotide in comparison with the corresponding wild type RNA polymerase.

10. (Twice Amended) The RNA polymerase of claim 1, wherein the modified wild type RNA polymerase has a further substitution, insertion or deletion of an amino acid other than the modification and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase activity.

11. (Amended) An RNA polymerase which is an RNA polymerase [derived] from T7 phage, and has tyrosine at amino acid residue 644 [or] and/or 667 of SEQ ID NO:2.

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Marked-up Claims 1, 3, 4, and 7-24

12. (Amended) The RNA polymerase of claim 11, wherein the RNA polymerase [derived] from T7 phage has a further substitution, insertion, or deletion of an amino acid other than the amino acid residues 644 [and] and/or 667 of SEQ ID NO:2, and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase activity.

13. (Amended) An RNA polymerase consisting of a wild type T7 RNA polymerase provided that 644th amino acid residue of SEQ ID NO:2 of the wild type T7 RNA polymerase, phenylalanine, has been replaced with tyrosine.

14. (Amended) An RNA polymerase consisting of a wild type T7 RNA polymerase provided that 667th amino acid residue, phenylalanine, of SEQ ID NO:2 of the wild type T7 RNA polymerase has been replaced with tyrosine.

15. (Twice Amended) The RNA polymerase of claim 13, wherein 665th amino acid residue, leucine, of SEQ ID NO:2 of the wild type T7 RNA polymerase has been replaced with proline.

16. (Amended) An RNA polymerase consisting of a wild type T7 RNA polymerase provided that 644th amino acid residue, phenylalanine, of SEQ ID NO:2 of the

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Marked-up Claims 1, 3, 4, and 7-24

wild type T7 RNA polymerase has been replaced with tyrosine, and 667th amino acid residue, phenylalanine, of SEQ ID NO: 2 of the wild type T7 RNA polymerase has been replaced with tyrosine.

17. (Amended) The RNA polymerase of claim 16, wherein 665th amino acid residue, leucine, of SEQ ID NO:2 of the wild type T7 RNA polymerase has been replaced with proline.

18. (Amended) An RNA polymerase which is an RNA polymerase [derived] from T3 phage, and has tyrosine at amino acid residue 645 or 668 of SEQ ID NO: 14.

19. (Amended) The RNA polymerase of claim 18, wherein the RNA polymerase [derived] from T3 phage has further substitution, insertion, or deletion of amino acid other than the amino acid residues 645 and 668 of SEQ ID NO:14, and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase activity.

20. (Amended) An RNA polymerase which is an RNA polymerase [derived] from K11 phage, and has tyrosine at one or more amino acid residues 664-669 and 690 of SEQ ID NO:15.

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Marked-up Claims 1, 3, 4, and 7-24

21. (Amended) The RNA polymerase of claim 20, wherein the RNA polymerase [derived] from K11 phage has a further substitution, insertion, or deletion of amino acid other than the amino acid residues 664-669 and 690 of SEQ ID NO:15, and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase activity.

22. (Amended) An RNA polymerase which is RNA polymerase [derived] from SP6 phage, and has tyrosine at one or more amino acid residues 633-638 and 670 of SEQ ID NO:16.

23. (Amended) The RNA polymerase of claim 22, wherein the RNA polymerase [derived] from SP6 phage has a further substitution, insertion, or deletion of an amino acid other than the amino acid residues 633-638 and 670 of SEQ ID NO:16, and wherein the further substitution, insertion, or deletion does not substantially affect the RNA polymerase activity.

24. (Twice Amended) A polynucleotide encoding at least [a part of] the RNA polymerase of claim 1.